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ELSEVIER SCIENCE FULL-TEXT ARTICLE

# p53 gene mutation analysis and antisense-mediated growth inhibition of human ovarian carcinoma cell lines.

**Skilling JS, Squatrito RC, Connor JP, Niemann T, Buller RE.**

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Targeting dysfunctional gene expression in the cancer cell with gene-specific therapeutics requires knowledge of the structure and expression of the designated gene. Because of the prevalence of p53 dysfunction in epithelial ovarian carcinoma, modulation of the expression of this tumor suppressor gene is an attractive target for gene therapy. We sequenced the p53 gene and analyzed its expression in 10 ovarian cancer cell lines. Only five cell line mutations were encountered, three associated with a loss of heterozygosity. Thus, neither p53 mutation nor allelic loss is required for ovarian carcinogenesis or propagation of ovarian cancer cell lines in vitro. SSCP screening, but not immunohistochemical staining, correlated with results of direct genomic sequencing. All p53 immunohistochemical-negative cell lines differed from that reported by another laboratory, underscoring the importance of the knowledge of target gene expression in a given cell line in a given laboratory. We designed pilot studies of antisense oligodeoxynucleotides directed against the p53 gene based on our sequence data. Differential growth inhibition of the A2780-CP-20 cell line (mutant p53 protein), but not of the OVCAR-3 cell line (wild-type p53 protein) confirmed the potential usefulness of this strategy.

PMID: 8557231 [PubMed - indexed for MEDLINE]

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1: Cancer Gene Ther 2000 Jan;7(1):4-12 

# Targeting gene expression to tumor cells with loss of wild-type p53 function.

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The tumor suppressor protein p53 is a transcription factor that can positively regulate the expression of critical target genes involved in negative control of cell growth or induction of apoptosis; p53 is also able to suppress the transcription of other genes by virtue of its ability to bind components of the basal transcription machinery. Over 50% of human tumors are characterized by p53 mutations that result in a loss of wild-type p53 (wtp53) function in the transcriptional control of these target genes. We have exploited this loss of p53 function in the regulation of gene transcription to develop a novel gene therapy strategy that maximizes expression of the potential therapeutic gene in tumors while simultaneously down-regulating the same gene in normal cells. In one construct (unit I), the potential therapeutic gene (in this case represented by a luciferase reporter) is placed under the control of a promoter such as the heat shock protein 70 gene promoter, which is repressed by wtp53 but overexpressed in many tumor cells with defective p53 function. Residual expression of the reporter in normal cells is repressed by cotransfection of another construct (unit II) consisting of a repressor of unit I under the control of a promoter that is activated by wtp53 expression. Unit II contains a promoter with a consensus wtp53 binding site driving a transcriptional repressor or an antisense construct for the gene in unit I. Our results suggest that this dual control approach may represent a strategy with wide applications in the field of cancer gene therapy.

PMID: 10678350 [PubMed - indexed for MEDLINE]

1: Nucleic Acids Res 2000 Jun 1;28(11):2234-41 Related Articles, Books, LinkOut

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# Loss-of-function genetics in mammalian cells: the p53 tumor suppressor model.

**Carnero A, Hudson JD, Hannon GJ, Beach DH.**

Institute of Child Health, London, UK.

Using an improved system for the functional identification of active antisense fragments, we have isolated antisense fragments which inactivate the p53 tumour suppressor gene. These antisense fragments map in two small regions between nt 350 and 700 and nt 800 and 950 of the coding sequence. These antisense fragments appear to act by inhibition of p53 mRNA translation both in vivo and in vitro. Expression of these antisense fragments overcame the p53-induced growth arrest in a cell line which expresses a thermolabile mutant of p53 and extended the in vitro lifespan of primary mouse embryonic fibroblasts. Continued expression of the p53 antisense fragment contributed to immortalisation of primary mouse fibroblasts. Subsequent elimination of the antisense fragment in these immortalised cells led to restoration of p53 expression and growth arrest, indicating that immortal cells continuously require inactivation of p53. Expression of MDM2 or SV40 large T antigen, but not E7 nor oncogenic ras, overcomes the arrest induced by restoration of p53 expression. Functional inactivation of both p21 and bax (by overexpression of Bcl2), but not either alone, allowed some bypass of p53-induced growth arrest, indicating that multiple transcriptional targets of p53 may mediate its antiproliferative action. The ability to conditionally inactivate and subsequently restore normal gene function may be extremely valuable for genetic analysis of genes for which loss-of-function is involved in specific phenotypes.

PMID: 10871344 [PubMed - indexed for MEDLINE]

1: Biochem Soc Trans 1996 Aug;24(3):410S 

# Antisense oligonucleotide-mediated inhibition of mutant p53 expression.

**Ruddell CJ, Green JA, Tidd DM.**

Department of Biochemistry, University of Liverpool, UK.

PMID: 8878954 [PubMed - indexed for MEDLINE]

1: Gynecol Oncol 1997 Jul;66(1):94-102 Related Articles, Books, LinkOut

ELSEVIER SCIENCE
FULL-TEXT ARTICLE

# p53 interference and growth inhibition in p53-mutant and overexpressing endometrial cancer cell lines.

**Janicek MF, Angioli R, Unal AD, Sevin BU, Madrigal M, Estape R, Averette HE.**

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BACKGROUND: The presence of p53 mutations and associated mutant p53 overexpression has been demonstrated in many cancer systems. Whether the overexpression of mutant p53 represents cause or effect, and whether p53 mutation contributes actively to the malignant phenotype is a matter of controversy. We examined the growth effects of oligonucleotides designed to interfere with p53 expression and/or activity in p53-mutant/overexpressing endometrial cancer cell lines. METHODS: Phosphorothioate oligonucleotides were used to target p53-related sequences in two p53-mutant/overexpressing endometrial cancer cell lines (KLE and RL95-2) and a normal fibroblast control. The ATP cell viability assay was used to measure growth effects after 6-day treatments with 27-mer and 14-mer sense (S) or antisense (AS) phosphorothioate oligodeoxyribonucleotides (oligos) targeting the promoter/ATG region of p53 and/or the p53 consensus (CON) DNA binding sequence. These sequences were designed to interfere with p53 expression and activity, respectively. Random sequences of the p53 27- and 14-mer were used as controls for nonspecific oligo effects, and a normal fibroblast cell line was used to compare oligo effects and serve as a negative p53 immunostaining control. RESULTS: Mean +/- SE IC50 (50% growth inhibition) of the S, AS p53, and p53 CON oligos were 4.2 +/- 1.3, 4.7 +/- 0.9, and 7.6 +/- 1.4 microM, respectively, for the two endometrial cell lines combined. The AS and S p53 oligos demonstrated dose-dependent inhibitory effects in both cell lines, while p53 CON produced variable effects alone and in combination with p53 AS. In KLE, a uniform inhibitory dose response was seen with p53 CON oligos. In RL95-2, the approximate IC50 for p53 CON was 0.5-1.0 microM, but at increasing doses above this, an inverse dose response was consistently observed. Combinations of p53 AS and p53 CON oligos produced predominantly synergistic growth inhibition. Although

combinations of p53 AS and p53 CON in KLE were synergistic at low doses, antagonistic effects occurred at higher concentrations. Oligos had little effect on normal fibroblast growth, with calculated IC50 > 16 microM. Equimolar combinations of p53 S and AS were antagonistic, indicating that antiproliferative effects were sequence-specific. Random oligos demonstrated some nonspecific inhibitory effects, with >25% growth inhibition at 16 microM and beyond. Immunoperoxidase staining for mutant p53 after exposure to 16 microM concentrations of p53 AS oligos demonstrated reductions in p53 staining but persistent overexpression relative to wild-type (fibroblast) cells. CONCLUSION: Phosphorothioate oligos directed against p53 sequences in two p53-mutant endometrial cancer cell lines demonstrated antiproliferative effects. Combined anti-p53 and anti-p53 binding site oligos resulted in predominantly synergistic antiproliferative effects. The activity of sense oligos, the variable responses to p53 CON, and the persistent overexpression of mutant p53 at high concentrations of growth-inhibiting anti-p53 oligos suggest that, while promising, the antineoplastic effects of these oligos occur through complex and incompletely understood mechanisms.

PMID: 9234928 [PubMed - indexed for MEDLINE]

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1: Cancer Res 1993 Oct 1;53(19):4452-5 

# Dominant negative effect of a germ-line mutant p53: a step fostering tumorigenesis.

**Srivastava S, Wang S, Tong YA, Hao ZM, Chang EH.**

Department of Pathology, Uniformed Services University of the Health Sciences, Bethesda, Maryland 20814-4799.

Lysates derived from the fibroblasts of individuals who are homozygous for normal p53 or heterozygous for the germ-line p53 mutation characteristic of certain Li-Fraumeni cancer-prone families were assessed for p53 function utilizing the binding of p53 protein to a p53-specific consensus oligonucleotide sequence. As expected, control nuclear lysates containing only mutant p53 or no p53 displayed little or no such binding. However, the nuclear lysates from heterozygous fibroblasts containing similar amounts of normal p53 and 245D mutant p53 displayed binding that was significantly below 50% of that seen with homozygous wild-type p53 in normal cell lysates. The nuclear lysates of these heterozygous or homozygous fibroblasts exhibited similar levels of DNA binding to a consensus oligonucleotide specific for the transcription factor, AP-1. These results indicate that mutant p53 has a transdominant effect on the binding of DNA by normal p53. These findings also suggest that p53 complexes formed in vivo that contain mutant p53 are functionally impaired even if normal p53 is also present in the complex. The implications of a trans-dominant effect of mutant p53 on the cancer-prone phenotype of individuals heterozygous for mutated p53 in Li-Fraumeni families is discussed.

PMID: 8402611 [PubMed - indexed for MEDLINE]

1: Biochem Biophys Res Commun 2001 Jan 12;280 (1):204-11

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ELSEVIER SCIENCE FULL-TEXT ARTICLE

# Dissociation of DNA binding and in vitro transcriptional activities dependent on the C terminus of P53 proteins.

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Wild type p53 protein requires posttranslational modification within a carboxy-terminal negative regulatory domain to activate DNA binding and transcription. Binding of monoclonal antibody PAb421 to the carboxy-terminal domain reproduces this activation. In the absence of PAb421, we found that wild type p53 bound actively to a template containing two copies of the p21WAF1p53 binding site. However, in an in vitro transcription assay with partially purified basal transcription factors, p53 only partially activated transcription from the same binding site and required PAb421 for full activation. Oncogenic missense mutant p53 proteins (N239 to S239, G245 to S245, R273 to H273) bound the WAF1 doublet significantly and were activated further by PAb421. However, these mutants were inactive in the transcription assay, even with PAb421. These results indicate that sequence-specific binding and transcriptional activities of p53 can be dissociated through C-terminal interactions and suggest that conformational changes induced by the mutations alter p53 interactions with basal transcription factors. Copyright 2001 Academic Press.

PMID: 11162500 [PubMed - indexed for MEDLINE]

1: Proc Natl Acad Sci U S A 1999 Aug 31;96 (18):10355-60



# The interaction between p53 and DNA topoisomerase I is regulated differently in cells with wild-type and mutant p53.

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DNA topoisomerase I is a nuclear enzyme involved in transcription, recombination, and DNA damage recognition. Previous studies have shown that topoisomerase I interacts directly with the tumor-suppressor protein p53. p53 is a transcription factor that activates certain genes through binding to specific DNA sequences. We now report that topoisomerase I can be stimulated by both latent and activated wild-type p53 as well as by several mutant and truncated p53 proteins in vitro, indicating that sequence-specific DNA-binding and stimulation of topoisomerase I are distinct properties of p53. These assays also suggest that the binding site for topoisomerase I on p53 is between amino acids 302 and 321. In living cells, the interaction between p53 and topoisomerase I is strongly dependent on p53 status. In MCF-7 cells, which have wild-type p53, the association between the two proteins is tightly regulated in a spatial and temporal manner and takes place only during brief periods of genotoxic stress. In marked contrast, the two proteins are constitutively associated in HT-29 cells, which have mutant p53. These findings have important implications for both cellular stress response and genomic stability, given the ability of topoisomerase I to recognize DNA lesions as well as to cause illegitimate recombination.

PMID: 10468612 [PubMed - indexed for MEDLINE]